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Full Length Research Paper

Genetic characterization of two traditional leafy vegetables (*Sesamum radiatum* Thonn. ex Hornem and *Ceratotheca sesamoides* Endl.) of Benin, using flow cytometry and amplified fragment length polymorphism (AFLP) markers

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Amplified fragment length polymorphism (AFLP) markers and flow cytometry were applied for the genetic characterization of wild and cultivated accessions of *Sesamum radiatum* and *Cerathoteca sesamoides*; two neglected and underutilized species of traditional leafy vegetable consumed in Benin. The average 2C nuclear DNA content per nucleus was found to be 1.99 ± 0.06 and 1.05 ± 0.06 pg for *S. radiatum* and *C. sesamoides*, respectively which correspond to estimated genome size of 1946,22 Mpb for *S. radiatum* and 1026,9 Mpb for *C. sesamoides*. No variation in DNA content could be detected within accessions from each analysed species. Also, no relation was found between nuclear DNA content, ecogeographical origin and the status (cultivated or wild) of the analyzed accessions. AFLP markers revealed low diversity within the accessions analyzed. Results from the study contributed to a better characterization of *S. radiatum* and *C. sesamoides* accessions and will help in defining both genetic resources conservation and breeding strategies.

Key words: Amplified fragment length polymorphism (AFLP) markers, *Ceratotheca sesamoides*, flow cytometry, genetic diversity, ploidy, *Sesamum radiatum*, leafy vegetables.

INTRODUCTION

The genus *Sesamum* belongs to the Pedaliaceae family. This family comprises about 16 genus and 60 annual and perennial species among which, *Sesamum* is the most important (Attibayéba et al., 2010). The genus is widely distributed in Africa, India and Brasilia. The most well-known species in this genus is *Sesamum indicum* which is an important oil crop. However, several other *Sesamum* species such as *Sesamum alatum* Thonn. ex Schumach, *Sesamum angolense* Welw., *Sesamum angustifolium* (Oliv.) Engl., *Sesamum radiatum* Thonn. ex Hornem.,

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Figure 1. Flowered branches of *C. sesamoides*.

Ceratotheca sesamoides (synonyme *S. heudoletii* Stapf – 1906) are distributed worldwide and consumed as leafy vegetables. In Benin, *S. radiatum* (Figure 1) and *Ceratotheca sesamoides* (synonyme *S. Heudoletii* Stapf - 1906; Figure 2) are widely distributed and play a significant role in the daily food and nutritional requirements of local people mainly in rural areas. Traditional leafy vegetables (TLVs) have long been known to contain fibres, minerals, vitamins and some have medicinal properties beside their nutritional values (Abukutsa, 2004; Francisca and Eyzaguirre, 2007; Dansi et al., 2008, 2009). *S. radiatum* and *C. sesamoides* are also known to cure infections and to facilitate digestion and childbirth (Konan et al., 2006). Despite their nutraceutical roles, they have been poorly researched and fall within the so called neglected and underutilised species (NUS) of Africa. For their sustainable conservation and utilisation, more investigations on their genetic diversity are prerequisite. To our knowledge, little information is available on the genetic characterization of *S. radiatum* and *C. sesamoides* using molecular markers [particularly amplified fragment length polymorphism (AFLP)] and flow cytometry. Chromosome numbers of 2n = 32 and 2n = 64 for *C. sesamoides* and *S. radiatum*, respectively were reported (Bedigian, 2004; Bedigian and Adetula, 2004). The presence of wild and cultivated forms of these species in some part of Benin (Adeoti et al., 2009) suggests the existence of some genetic variability which could be related to edaphoclimatic conditions.

Most studies on plant genetic diversity are now based on the use of molecular markers among which is, amplified fragment length polymorphic DNA (AFLP) which has become one of the molecular markers of choice in genetic diversity analysis due to its capability to disclose a high number of polymorphic markers by single reaction (Vos et al., 1995). AFLPs were described as a powerful and efficient approach in population genetics and diversity analysis, molecular taxonomic classification, gene mapping and marker-assisted breeding for many crop species (Carr et al., 2003; Uptmoor et al., 2003). It provides an effective means of covering large areas of a plant genome in a single assay (Zhang et al., 1999; Muminovic et al., 2004). It is highly reproducible and discriminative (Soleimani et al., 2002) and generates a virtually unlimited number of genetic markers (Gaudeul et al., 2000). AFLP has been already used to assess genetic diversity in many crop species such as peach tree (Xu et al., 2006), linseed (Adugna et al., 2006), soybean (Tara et al., 2006), wheat (Shoaib and Arabi, 2006), sesame (Hernan and Petr, 2007) and fonio (Adoukonou-Sagbadja et al., 2007).

In molecular cytogenetic study in plant, flow cytometry

Figure 2. Flowered branches of *S. radiatum.*

remains the mostly used approach. It is a rapid and efficient method to determine ploidy level (Lysák and Doležel, 1998; Emshwiller, 2002), estimate nuclear DNA content and genome size (Lysák et al., 2000) and ploidy level in plants. It offers quickness, precision and accuracy in detecting small differences in DNA content of large numbers of nuclei (Dolezel, 1997) and do not require large quantities of plant material. For many purposes, flow cytometry techniques have been applied to several plants including rice (Cesar et al.,1994), wheat (Pfosser et al.,1995), *Pisum sativum* (Neuman et al., 1998), fonio (Adoukonou-Sagbadja et al. 2007) and tomato (Brasileiro-vidal et al., 2009).

The objectives of this study were three fold: (i) To determine the ploidy level and the nuclear DNA content of various accessions of *S. radiatum* and *C. sesamoides* collected in Benin; (ii) Examine the relationship between genome size and the ecogeographical distribution of the species; (iii) Assess the genetic diversity within accessions of these species using AFLP markers

MATERIALS AND METHODS

40 accessions of *S. radiatum* and eight of *C. sesamoides* were used for flow cytometric analysis. For AFLP analysis, 40 accessions of *S. radiatum* and 30 of *C. sesamoides* were used. Samples of *S.*

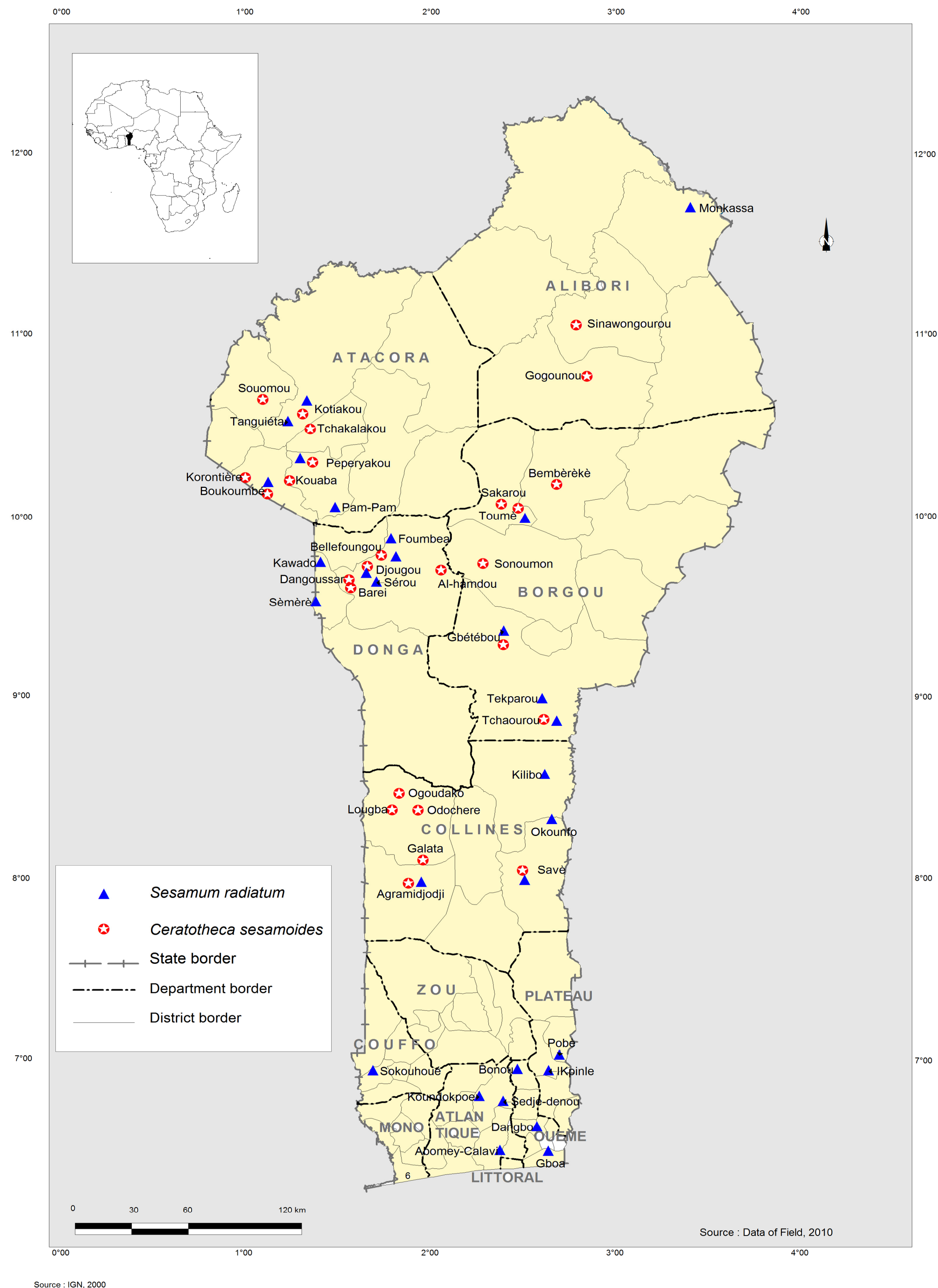

Figure 3. Collecting sites of *S. radiatum* and *C. sesamoides* accessions.

radiatum were collected from the laboratory nursery for cultivated species and wild accessions were gathered from the fields. All accessions of *C. sesamoides* were gathered from their natural biotope in Benin (Figure 3). Fresh plant material was preserved in moistened paper, enclosed in plastic bags and brought to the laboratory for analysis.

Nuclei preparation

Leaves from both plant species gave very sticky extracts which made the preparation of nuclei difficult. Young stems sampled on six week-old seedlings were used for cytometric analysis. In order to properly release nuclei, 1 ml of extraction buffer was added to

fresh plant material and young stems were chopped together with the leaves from the standard species *Petunia hybrida* (2C = 2x = 2.85 pg; 41.0% GC; Marie and Brown, 1993) in a sterile Petri dish using a sharp razor blade. Dolezel's buffer supplemented with metabisulfite (5 mM), polyvinyl pyrolidone (PVP, 1%) and RNaseA (50 µg/ml) was used for nuclei extraction, according to Dolezel and Bartos (2005). The resulting suspension was filtered and nuclei were stained with a propidium iodide (IP) solution to a final concentration of 50 µg/ml.

Flow cytometric measurements

DNA content of the nuclei was measured by relative fluorescence of samples with a PARTEC CyFlow SL 532 nm cytometer. Measurements were performed on FL2 area histograms. At least, 10000 nuclei per sample were analysed. Estimation of nuclear DNA content was performed using nuclei from *P. hybrida* (2C = 2.85 pg) as an internal standard. Then the 2C nuclear DNA content of the unknown sample was calculated according to the formula: Sample 2C DNA content (pg) = (sample peak mean/standard peak mean) x 2C DNA content of standard. Conversion of DNA content into base pairs was done according to the factor 1 pg = 978 Mbp (Dolezel et al., 2003).

Extraction of genomic DNA

Fresh leaves (ca 0.5 g FW) were deep frozen in liquid nitrogen then ground into powder with mortar and pestle. The powder was mixed with preheated (50°C) extraction buffer solution A containing CTAB (2 g); Tris pH = 8 (1 M; 1 ml); EDTA.Na_2 (0.5 M; 20 ml); NaCl (5 M; 28 ml); PVP 40000 (1 g); sodium bisulfite (1 g) and H_2O Qsp 100 ml. The mixture was incubated at 65°C for 30 min. After cooling, 5 ml of chloroform isoamyl alcohol (24:1) were added and the extract was homogenized for 10 min at room temperature. The homogenate was then centrifuged for 20 min at 13000 g at room temperature. The supernatant was transferred into a new tube (volume 13 ml) and 200 µl of poteinase K (2 µg/µl) was added and incubated for 1 h at 37°C. Then 500 µl of preheated (50°C) solution B [CTAB (1g); NaCl (5M; 1.4 ml); H_2O qsp 10 mL] and 5 ml of chloroform isoamyl alcohol (24:1) were added and the mixture was carefully homogeneised for 10 min at room temperature. Then, the homogenate was centrifuged for 10 min at 4500 rpm at room temperature. The extract was transferred into a new tube (type Falcon 50 ml) and 10 ml of solution C were added [CTAB (1 g); Tris pH = 8 (1 M, 5 ml); EDTA.Na_2 (0.5 M, 20 ml); H_2O Qsp 100 ml], followed by a 15 min-centrifugation at 20000 g. The supernatant was discarded and the pellet was dissolved with 1 ml of solution D [Tris pH = 8 (1M, 0.1 ml); EDTA.Na_2 (0.5 M; 200 ml); NaCl (5M, 2 ml); H_2O qsp 10 ml] and incubated at 65°C for 30 min. Then, 800 µl of cooled isopropanol was added and the tube was gently stirred until precipitation of DNA. After centrifugation at top speed for 10 min, the supernatant was discarded and the pellet was suspended in 500 µl of sterile water. A final purification of the obtained DNA solution was undertaken using filtration through a silica membrane following the protocol. Ten microlitres of Rnase (1 µg/µl) was added to the DNA solution following 30 min of incubation at 37°C. Then, 50 ml of NaCl (5M), 150 µl of AcK (60 ml of AcK 5M, 11.5 ml icy acetic acid (37%) and 28.5 ml of H_2O), 900 ml of guanidium chloride/ethanol (7.8 M / ethanol: 1/3 of guanidium chloride + 2/3 of ethanol 96%) were added to 200 µl of DAN solution and incubated for 10 min at room temperature. 750 ml of the homogenate was applied into the fixation plate [plate 96 Whatman Unifilter 800 GF/B (ref 7700-2803)] and centrifuged at 6000 rpm for 2 min. This step was repeated two times. The liquid was discarded and 600 ml of washing buffer [100 ml of mixture (160 mM of AcK; 22.5 mM of Tris HCl pH 8; 0.1 mM of EDTA) + 170 ml of ethanol 96%] was applied on the column and the plate was centrifuged at 600 rpm for 10 min. The liquid was discarded and this step was repeated one again for the remaining mixture. Afterwards, 100 ml of preheated (65°C) ultra pure water was applied on the column for 5 min. Then, the plate was centrifuge at 6000 rpm for 2 min and the DNA liquid was recovered.

AFLP protocol

AFLP analysis was performed as originally described by Vos et al. (1995) with minor modifications. In this study, 250 ng of genomic DNA (10 µl of working solution) were digested using *Eco*RI and *Mse*I restriction enzymes and the generated fragments were ligated with double-stranded site-specific adapters using T4 DNA ligase. Following ligation, a pre-amplification was carried out with primers containing one selective nucleotide, namely cytosine and adenine for *Mse*I and *Eco*RI primers, respectively. PCR was performed for 30 cycles (1 min at 94°C, 1 min at 56°C, and 1 min at 72°C) with a final extension step of 3 min at 72°C. Resulting PCR products were diluted 10 times with sterile water and used as templates for the selective amplification step. This was carried out with a couple of selective primers (EcoRI/MseI) carrying three selective nucleotides at their side. EcoRI was labelled with fluorescent dye. Selective amplification was performed on two steps in a total volume of 20 µl containing 5 µl of diluted pre-selective PCR product, 2 µl of each primer at 1 pmole/µl for EcoANN and 5 pmole/µl for MseCNN and 0.2 µl of 1 U of Taq polymerase. The first step of selective amplification consisted of 12 cycles (3 min at 94°C, 45 s at 94°C and 45 s at 65°C) and 1 min at 72°C for final extension. The second step was performed for 25 cycles which consisted of 45 s at 94°C, 45 s at 56°C, 1 min at 72°C and 3 min at 72°C. The PCR products resulting from selective amplification were diluted 10 times and an aliquot (2 µl) of diluted solution was mixed with 18 µl of a ROX-labeled internal size standard (AMM 524). The mixture was then denaturised for 5 min at 95°C, loaded and separated on an ABI PRISM 3130X Genetic Analyzer sequencer (Applied Biosystems).

Flow cytometry analysis

Nuclear DNA content data were analyzed using SPSS v. 16.0 Software. One way analysis of variance (ANOVA) was used to test the differences between DNA content of accessions in relationship with ecogeographic origin and the least significant difference test was used to locate significance within ANOVA.

Scoring and analysis of AFLP data

Electrophoregrams generated by the sequencer were analysed using the GeneMapper 3.7 software package (Applied Biosystem, 2004). Clear and unambiguous peaks were considered as AFLP markers and scored as present (1) or absent (0) in order to generate a binary data matrix. The total number of scored markers and the number and percentage of polymorphic markers were determined for each primer pair used. With the binary matrix (0, 1) compiled, pairwise relatedness between all accessions was estimated using Dice's index (Dice, 1945). Using NTSYS- pc software package 2.01, similarity matrix was submitted to cluster analysis using the UPGMA method in order to bring out the relationship between accessions (Rohlf, 2000).

RESULTS AND DISCUSSION

Genome size and ploidy level

An example of histograms generated by flow cytometric

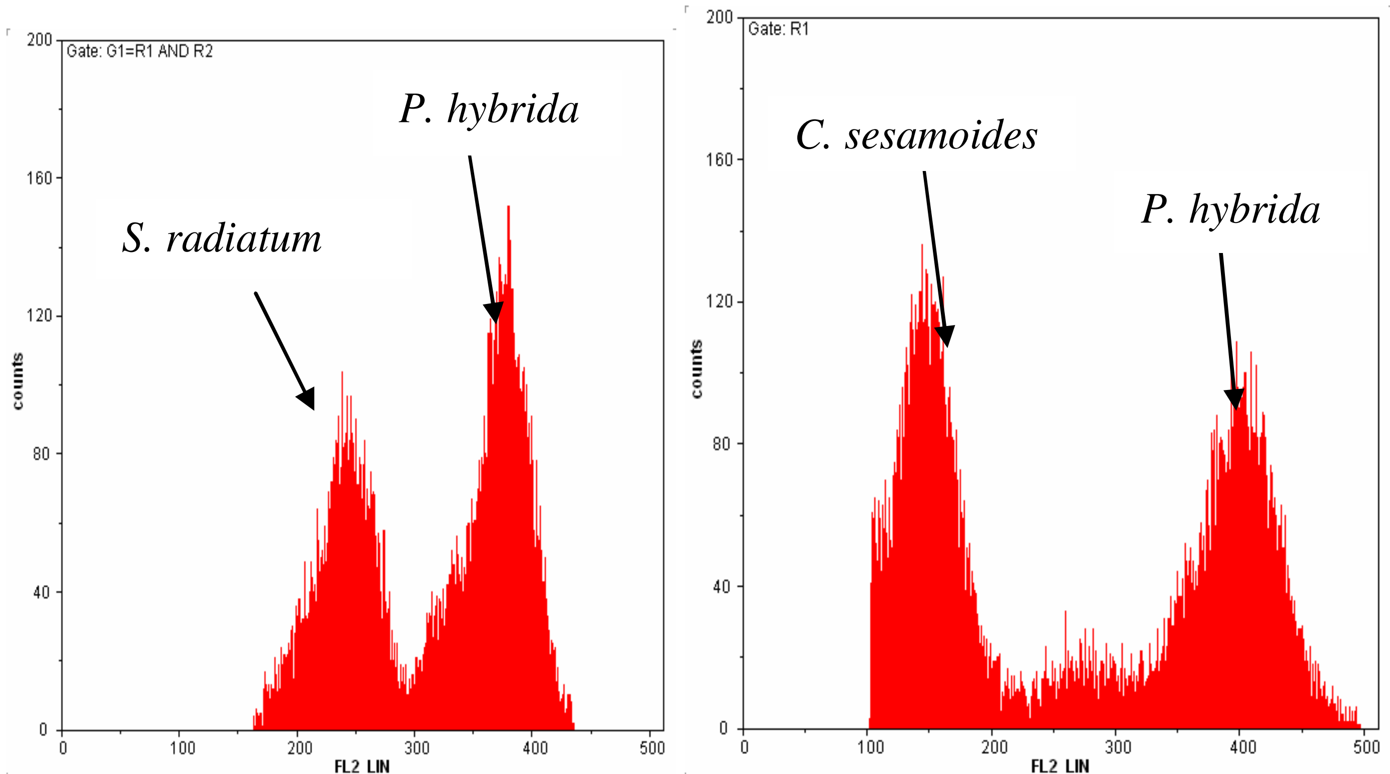

Figure 4. Histogram of the relative nuclear DNA content of *S. radiatum* and *C. sesamoides* using *P. hybrida* as internal standard.

analysis is presented in Figure 4. Flow cytometric analysis generated a single peak corresponding to the 2C level. Peaks corresponding to the 4C level were not detected, thus indicating that there were very few cell divisions occurring in young stems at the time of sampling. Moreover, no polyploid material could be detected among all analysed accessions.

The nuclear DNA content, origin and status of each accession are shown in Tables 1 and 2. The relative nuclear DNA content within *S. radiatum* accessions (Table 1) ranged from 1.82 to 2.10 pg per nucleus (average value = 1.99 pg ± 0.06). Nevertheless, the 2C nuclear DNA content estimated within various accessions were found to be not statistically different. In *C. sesamoides* accessions (Table 2), nuclear DNA content ranged from 1.01 to 1.09 pg/nucleus (average value = 1.05 ± 0.06 pg). The average DNA content of *S. radiatum* was found to be slightly lesser than two-fold the one measured in *C. sesamoides*. Indeed, the chromosome numbers for *S. radiatum* and *C. sesamoides* are respectively 2n = 64 and 2n = 32 (Nakamura and Sato, 1956; Bedigian, 2004; Bedigian and Adetula, 2004). The great difference in nuclear DNA content observed between the two species under study could be explained by their phylogenetic distance and highly support their botanical classification in two distinct genera. Similar results were reported in *Digitaria* sp. landraces where nuclear DNA amounts of the two wild species *Digitaria ciliaris* (Retz.) Koeler and *Digitaria lecardii* (Pilg.) Stapf. classified in other sections of the genus *Digitaria*, were 1,3 to 1,4 fold higher than that observed for cultivated species (Adoukonou-Sagbadja et al., 2007). The 2C DNA content we have measured in *S. radiatum* differ from the one reported by Hiremath et al. (2001) using Feulgen microspectrophotometry method. In contrast, it is worth noting that flow cytometry and Feulgen microspectrophotometry analysis when applied for the determination of nuclear DNA content of *Capsicum* species provided very similar values (Edouardo et al., 2003). However, it has been reported that variation in DNA content could often relate to polyploidy, aneuploidy, variation in the amount of heterochromatin, occurrence of supernumerary chromosomes (B chromosomes), deletion or duplication of chromosomes segments and variation in the copy number of repeated sequences (Poggio et al., 1998; Achigan-Dako et al., 2008). According to Dolezel and Bartoš (2005), the precise detection of intraspecific variation in genome size is not a trivial task even if genome size variation were reported in species such as *Arabidopsis thaliana* (L.) Heynh. (Schmuths et al. 2004) and *Festuca pallens* Host (Šmarda and Bures, 2006).

Environmental factors may also have an important impact on genome size. Altitude and latitude were used as proxies for abiotic influences putatively acting on genome size (Achigan-Dako et al., 2008). Positive, negative or polynomial relationships have been described

Table 1. Relative DNA content revealed with *S. radiatum* accessions.

Accession number	Collecting site	Status	2C DNA content (pg)	
			Mean ± SD	CV
Sr01	Abomey-calavi	Wild	1.95 ± 0.00	0.03
Sr02	Atanwignan I	Wild	2.02 ± 0.03	1.70
Sr03	Atanwignan II	Wild	2.10 ± 0.00	0.18
Sr04	Bellefoungou	Cultivated	1.88 ± 0.03	1.50
Sr05	Bonou	Wild	2.07 ± 0.02	0.96
Sr06	Cotiakou	Cultivated	1.94 ± 0.03	1.33
Sr07	Dangbo	Wild	1.95 ± 0.11	5.45
Sr08	Djougou	Cultivated	1.95 ± 0.03	1.54
Sr09	Foli	Wild	2.09 ± 0.02	1.18
Sr10	Foumbéa	Cultivated	2.03 ± 0.02	0.78
Sr11	Gbétébou I	Cultivated	2.00 ± 0.01	0.50
Sr12	Gbétébou II	Cultivated	1.97 ± 0.04	1.78
Sr13	Gboa	Wild	2.07 ± 0.02	0.81
Sr14	Houébossou I	Wild	2.05 ± 0.02	1.00
Sr15	Houébossou II	Wild	1.99 ± 0.06	2.98
Sr16	Hounti	Wild	1.98 ± 0.04	1.99
Sr17	Ikpinlè	Wild	1.96 ± 0.02	1.04
Sr18	Kawado	Cultivated	1.95 ± 0.03	1.61
Sr19	Kilibo	Cultivated	1.91 ± 0.04	2.12
Sr20	Koudokpoé	Wild	1.95 ± 0.05	2.58
Sr21	Koupagou	Cultivated	2.00 ± 0.01	0.67
Sr22	Monkassa	Wild	1.82 ± 0.05	2.52
Sr23	Okounfo	Cultivated	1.94 ± 0.01	0.33
Sr24	Pam Pam	Cultivated	2.01 ± 0.04	2.07
Sr25	Péporiyakou	Cultivated	1.99 ± 0.08	4.28
Sr26	Pobè	Wild	1.96± 0.01	0.30
Sr27	Savè	Cultivated	1.98 ± 0.07	3.60
Sr28	Sèdjè-Dènou	Wild	2.00 ± 000	0.23
Sr29	Sèmèrè	Cultivated	1.92 ± 0.03	1.44
Sr30	Sérou	Cultivated	2.06 ± 0.05	2.42
Sr31	Sokounhoué I	Wild	2.03 ± 0.04	1.77
Sr32	Sokounhoué II	Wild	2.01 ± 0.04	2.07
Sr33	Tanguiéta	Cultivated	2.01 ± 0.04	1.95
Sr34	Tchaourou I	Cultivated	2.01 ± 0.01	0.36
Sr35	Tchaourou II	Cultivated	2.01 ± 0.02	0.85
Sr36	Tékparou	Cultivated	1.95 ± 0.01	0.56
Sr37	Toumè I	Cultivated	1.97 ± 0.09	4.52
Sr38	Toumè II	Cultivated	1.90 ± 0.07	3.61
Sr39	Toumè III	Cultivated	1.97 ± 0.01	0.39
Sr40	Toumè IV	Cultivated	2.03 ± 0.03	1.47
Mean value			1.99 ± 0.06	2.96

(Knight et al., 2005). Indeed, effects of a geographical gradient on DNA content in cultivated maize, *Festuca pallens*, *Arabidopsis thaliana* and *Lagenaria siceraria* (Mol.) populations sampled at different altitudes have been reported (Bennett, 1985; Poggio et al., 1998; Šmarda and Bures, 2006; Schmuths et al., 2004; Achigan-Dako et al., 2008). In this study, no correlation between DNA content and the geographical origin of accessions could be observed. In *S. radiatum* which was found both in the wild and as a cultivated plant, the analysis of variance based on the geographical origin or the status of accession clustered all the acessions in one single group. The homogeneity of the group was indicated by a non-significant F-value (F = 0.836) intra-

Table 2. Relative DNA content revealed with *C. sesamoides* accessions.

Accession number	Collecting site	Statu	2C DNA content (pg)	
			Mean ± SD	CV
Cs01	Yataninga	Wild	1.02 ± 0.00	0.44
Cs02	Odochere	Wild	1.04 ± 0.02	1.92
Cs03	Barei	Wild	1.05 ± 0.03	2.95
Cs04	Koutadjèbou	Wild	1.01 ± 0.09	8.91
Cs05	Dangoussar	Wild	1.09 ± 0.022	0.012
Cs06	Sakarou	Wild	1.07 ± 0.02	2.12
Cs07	Sinawongourou	Wild	1.05 ± 0.02	1.71
Cs08	Kassakpéré	Wild	1.05 ± 0.03	2.39
Mean value			1.05 ± 0.06	5.46

Table 3. Number of AFLP amplicons and corresponding rate of polymorphism for the two species under study.

Species	Primer combination	Number of amplicon	Number of polymorphic amplicon	Percentage of polymorphism
S. radiatum	Eco AAG/ Mse CTC	104	16	15
	Eco ACG/ Mse CAG	214	27	13
	Eco ACG/ Mse CTA	111	17	15
	Eco AGG/ Mse CTC	64	3	5
C. sesamoides	Eco ACT/ Mse CAT	113	35	31
	Eco AAG/ Mse CTC	52	20	38
	Eco ACG/ Mse CAG	59	22	37

specific variation within accessions of each species.

These results indicate a noticeable stability of the genome size of both species, independently of the geographical origin of accessions. Genome size uniformity has been already reported in many crops such as soybean (Greilhuber and Obermayer, 1997) and groundnut (Temsch and Greilhuber, 2000). However, a more detailed analysis is needed which will integrate multiple parameters such as rainfall, soil type and temperature in order to properly monitor the relationship between genome size and ecological distribution of both *S. radiatum* and *C. sesamoides*.

Estimation of intraspecific genetic diversity

To our knowledge, this study is the first attempt to apply AFLP markers to the study of genetic diversity in *S. radiatum* and *C. sesamoides*. Of the nine primers combinations used for both species, four and three combinations showing reliable PCR results respectively for *S. radiatum* and *C. sesamoides* were selected for use on the full set of samples. For *S. radiatum*, 493 amplicons were obtained among which 63 were found to be polymorphic. The percentage of polymorphic amplicons generated by each primer combination ranged from 3 to 27%. The average of polymorphic amplicons in *S. radiatum* accessions reached 12.77%. The genetic similarity index calculated using Dice's index varied from 0.15 to 0.85 in this species.

In *C. sesamoides* samples, a total of 224 amplicons was obtained, from which 77 were found to be polymorphic. The percentage of polymorphic amplicons was 34.37% and this value ranged from 20 to 35%, depending on the primer combination. The genetic similarity index calculated using Dice's index ranged from 0.16 to 0.82. The variation of genetic similarity within *S. radiatum* and *C. sesamoides* accessions is approximately the same. The high level of genetic similarity obtained for both species indicated a rather low intraspecific genetic diversity (Table 3). Likewise for *S. radiatum*, the generated dendrogram based on genetic distance using the neighbor-joining approach of the UPGMA method clustered accessions independently from their geographical origin and status (wild or cultivated). For a genetic similarity value of 0.66, three different clustering groups were found (Figure 5). Groups I and II clustered both cultivated and wild accessions. Besides, these clusters also grouped together accessions from both northern and southern parts of the country. The third group was made of one single wild accession sampled at Sèdjè-Dènou, a village located in the southern part of Benin. Indeed, our results indicate a high degree of genetic similarity between wild and cultivated accessions.

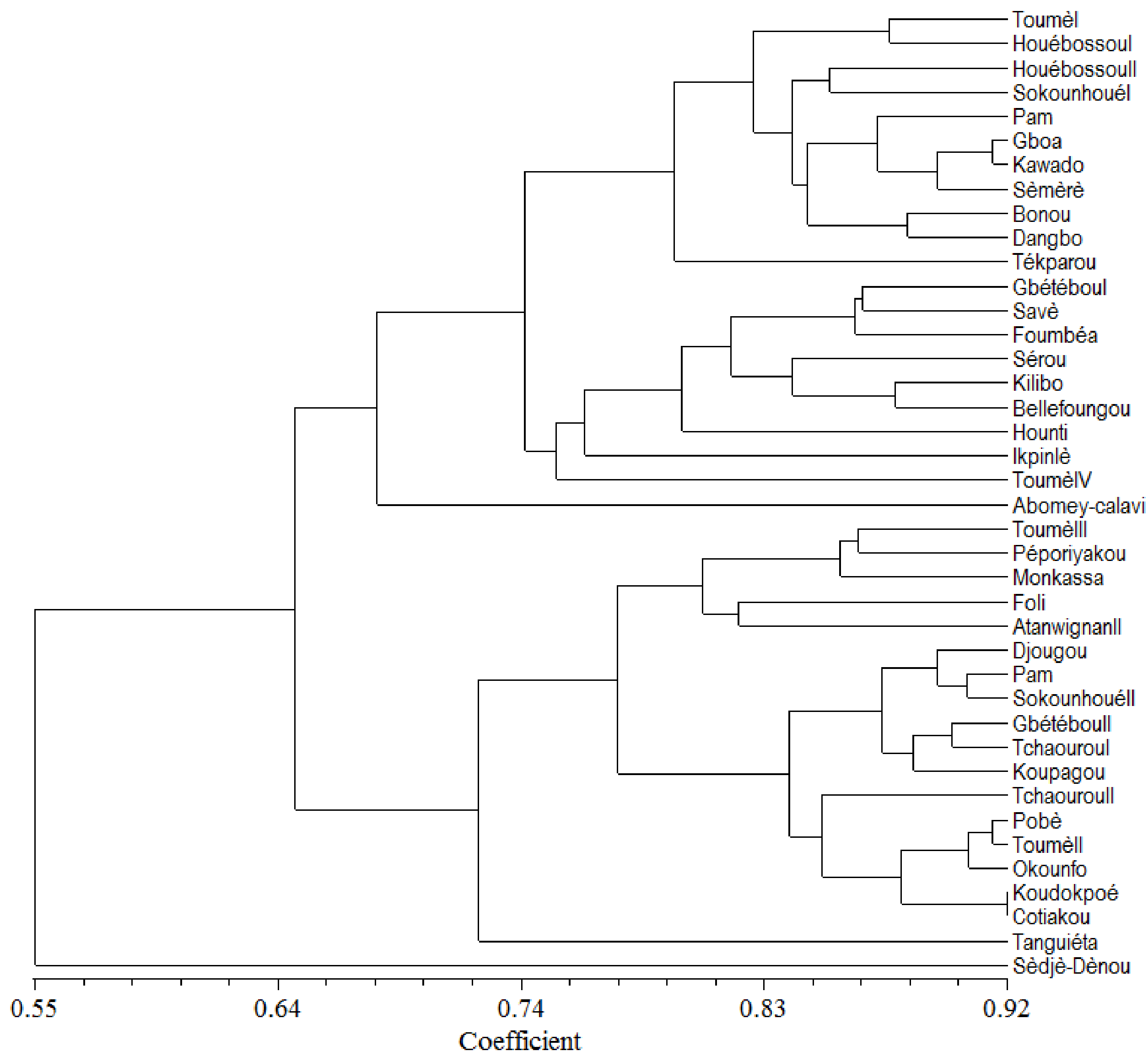

Figure 5. Dendrogram generated for *S. radiatum* accessions using UPGMA cluster analysis based on Dice's index estimates for AFLP data.

No correlation could be found between the geographical distribution and the clustering of accessions revealed by AFLP analysis (Figures 5 and 6). Possible occurrence of seed exchanges via producers and local markets may account for this low genetic diversity. Similar results were reported for *S. indicum* in Central Asia and they were explained by the exchange of sesame seeds between very distant regions (Kim et al., 2002). In contrast, a high genetic variability was reported for sesame accessions from Turkish, China, Japan and India (Kim et al., 2002; Ercan et al., 2004). The distribution of genetic diversity for a given plant depends not only on intrinsic characters resulting from its evolution, such as its reproductive biology but also on ecological and geographical factors and often on human activities (Ramanatha and Hodgkin, 2002). When a long distance exists between accessions and a narrow genetic diversity is detected, it is probable that few genetic changes have occurred during the evolution and diversification process of the species. According to Tiang et al. (2003), for a wide range of plant species, the mating system plays a critical role in the determination of the patterns of genetic variation both within and between populations. In our study, both species use only sexual reproduction. *S. radiatum* and *C. sesamoides* are autogamous and such species are known for their low intraspecific and high interspecific diversity (Hamrick et al., 1991). Nevertheless, apomixis, an asexual propagation through seeds, contributed to genetic fixation (Berthaud, 2001; Hörandl and Paun, 2007) with a lower genetic diversity among populations

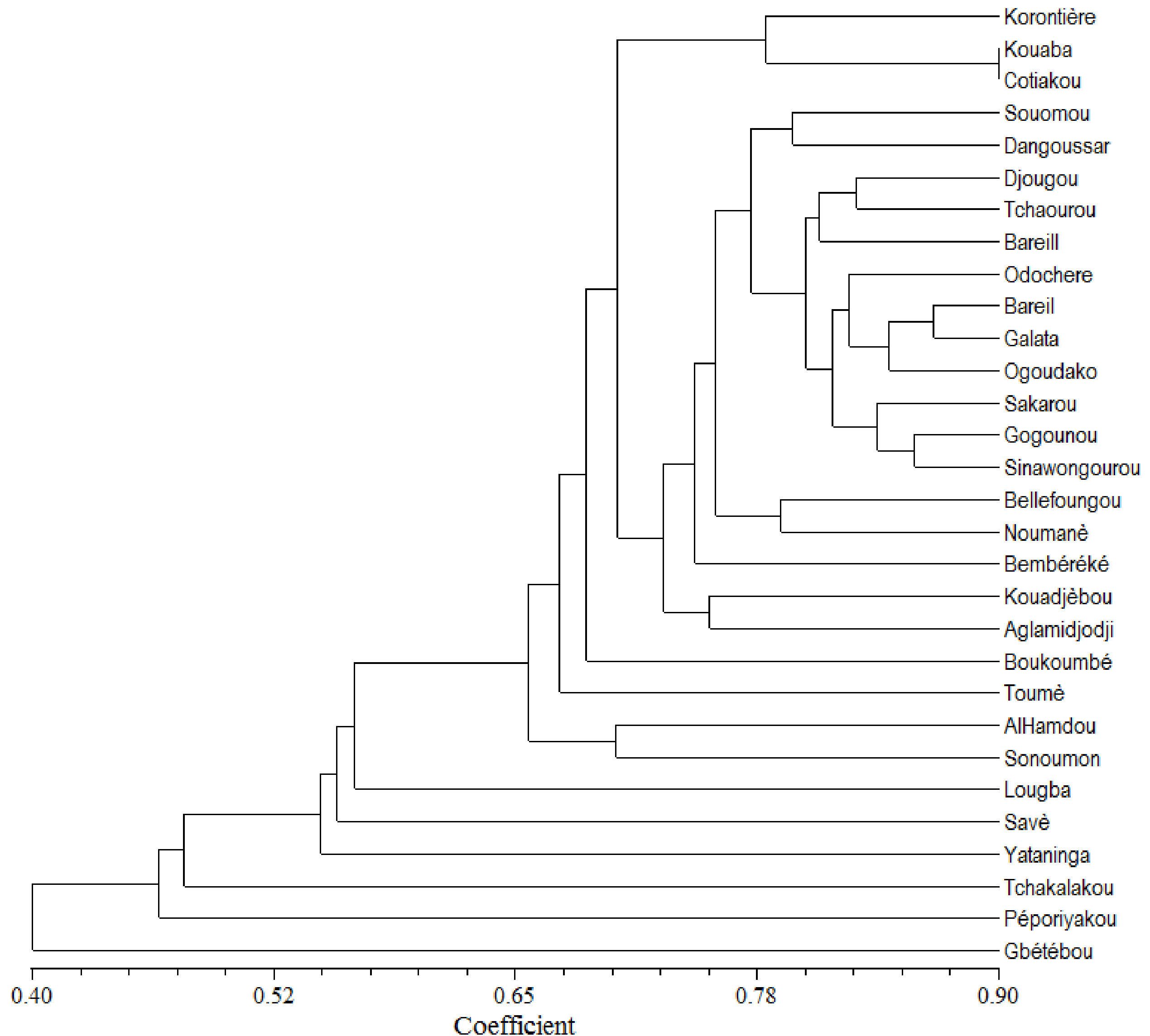

Figure 6. Dendrogram generated for *C. sesamoides* accessions using UPGMA cluster analysis based on Dice's index estimates for AFLP data.

where it occurred. Then it constitutes a lagging factor for genetic recombination. However, genetic diversity has been reported within apomicts species such as *Tripsacum* (Berthaud, 2001), mangousteen *Garcinia mangostana* L. (Ramage et al., 2004) and *Paspalum dilatatum* Poir. (Miz and de Souza-Chies, 2006). Then apomixis might be an explanation for the paradox of such a narrow genetic diversity in a species which is strictly propagated through sexual reproduction. However, the existence of such a phenomeneon in *Sesamum* still needs to be evidenced. Moreover, the sample size is small for investigating adequately a possible correlation of genetic diversity with ecogeographic origin of the species accessions. Therefore, it will be useful to increase the sample size through seeds collection survey to better assess the genetic diversity of the two species.

Conclusion

Our results on the genetic characterization of *S. radiatum* and *C. sesamoides* clearly indicate a great stability in genome size and a low intraspecific genetic diversity. No genetic structuration and genome size variation could be found among accessions studied. In the future, the development of more specific and discriminative molecular markers such as microsatellites will facilitate a more precise analysis of intraspecific genetic diversity. More research work is needed in order to confirm the impact of pedoclimatic parameters on the genome size,

and to elucidate the possible role of long distance pollinator in the dispersal of seeds. Widening of study area to the bordering countries (Niger, Nigeria and Togo) where the species are also consumed could lead to better understanding of the genetic diversity and the mode of propagation of the two species.

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